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(54) Title: EXTRACTION OF NUCLEIC ACIDS

(57) Abstract: A method of isolating nucleic acids from liquid mixtures comprises passing an electric current through the liquid so as to form a positively charged surface to which the nucleic acids become bound and from which they can subsequently be removed.

Extraction of Nucleic Acids

The present invention relates to a method of isolating nucleic acids, particularly DNA, from biological mixtures.

The isolation and amplification of nucleic acids is used in many applications, for example in the detection and diagnosis of genetic and infectious disease, in forensic medicine, in the identification of new genes or allelic variations or mutations and in aiding routine genetic manipulation e.g. sequencing.

Amplification techniques such as polymerase chain reaction (PCR) are widely used and have proved very useful in a range of applications, however PCR cannot be used directly on clinical samples as the samples contain substances which inhibit the amplification enzyme and the presence of red blood cells presents a particular problem.

In order to overcome this problem the nucleic acids are isolated from the samples before amplification etc.

A known method in which nucleic acids may be separated by charge differences uses gel electrophoresis. This is performed by adding the sample to a well in the gel and applying a voltage e.g. 100 volts, 60amps, through the gel, the nucleic acids gradually migrate through the gel depending on their molecular size. This method of separation is intended for analytical techniques relating to molecular size rather than extraction, since the nucleic acids are retained in the gel and would require further purification.

The present invention provides an improved method for separating of nucleic acids by electrical charge without using a gel.

According to the invention there is provided a method for separating nucleic acids from liquid mixtures containing them which method comprises forming a positively charged surface in the liquid and forming an electric field through the liquid to cause nucleic acids to migrate towards the positive charged surface and binding the nucleic acids to the positively charged surface.

The invention also provides a method for separating nucleic acids from liquid mixtures containing them which method comprises passing an electric current through the liquid to form a positively charged surface in contact with the liquid to cause nucleic acids to migrate towards the positive charged surface and binding the nucleic acids to the positively charged surface.

The positively charged surface is formed, to which the nucleic acids migrate and become bound so that, after period of time, the surface can be removed and the nucleic acids separated.

The nucleic acids, having a negative charge at physiological pH e.g. above pH 5 for example pH 5 to 9, will be attracted to a positively charged surface, and preferably the liquid is preferably buffered to maintain it at physiological pH. After washing away contaminants, the nucleic acids can be removed from this surface for analysis using PCR or other techniques. The nucleic acids can be released by turning off the current or reversing the polarity or by washing.

The nucleic acids bind to the positively charged surface by virtue of their negative charge, and the force binding them to the surface can be the electrical attraction between the negatively charged nucleic acids and the positively charged surface.

In one embodiment of the method of the invention a sample containing cells is diluted in a lysing reagent that releases the nucleic acids into a liquid and a current or

potential difference (i.e. an electric field) is then applied through the liquid using a technique in which a surface with a positive charge is generated.

When an electric field is generated across the tube or vessel containing the liquids in which there are the nucleic acids, the nucleic acids will then move to the sides of the tube or vessel according to the local flux. The electric field can be applied from outside the tube or vessel.

An electric current can be generated by having electrodes in contact with the liquid and the voltage can be in the order of 20 volts e.g. 5 to 50 volts so that an electric field is set up between the electrodes.

The electrodes can be in any convenient form, for example they can be made from an inert metal or conductive plastics material such as a plastic containing conductive particles such as graphite. This electrode can be made from an inert plastics material such as a polypropylene. The conductive material can be incorporated in a filter, membrane or pipette tip etc. through or across which the liquid containing the nucleic acids is passed so that the nucleic acids become bound to the filter, membrane or pipette tip etc.

An electrode e.g. metal electrode can be covered with a porous plastic material such as dialysis tubing so that the liquid forms a conductive path through the tubing, the nucleic acids then migrate to and become bound or attached to the tubing whilst the electric current is flowing and the bound nucleic acids can subsequently be removed.

In an embodiment of the invention a pipette incorporates a conducting tip through which an electric current can be conducted, in this case the conducting pipette tip can be used to bind DNA by either having a two pipettes in the same sample vessel or tube and passing a current between them, so the nucleic acids will become bound to the positively charged pipette tip. Alternatively the sample tube can be modified e.g.

it is modified with metal electrodes or the sample tube has conducting material incorporated in the tube itself so that current is passed through liquid with the pipette tip as the positive electrode.

For very small samples, where a small quantity of nucleic acids is present, e.g. viral RNA from serum, a conducting material may be included in a PCR tube or plate such as the wells in a 96 hole plate. If the PCR tube or plate is made positive relative to a negative electrode or tip dipping in the solution containing the nucleic acids, the nucleic acids will bind to the tube walls or plate, allowing the contaminants to be washed away. The bound nucleic acids may then be released by switching the charge off and the DNA is ready for PCR in the same tube.

Thus the invention can be performed in a variety of formats for large or small samples and in an automated system.

The invention is particularly useful for separating nucleic acids from liquids which contain other material such as cell debris obtained from lysing cells, etc. which is not negatively charged.

One embodiment of the invention is the isolation of DNA from whole blood as described in the example.

Example

3ml of whole blood was mixed with 30ml of 10mM Ammonium bicarbonate and 5% Tween 20 with 10mM Tris HCl pH 9, proteinase K 200 µg/ml. To form a buffered solution containing nucleic acids lysed from the blood cells. Conductive electrodes made of copper were covered with dialysis tubing containing the same buffer without the protease and dipped into the diluted sample. A power unit was used to apply a voltage of 20V between the electrodes and then left to run for 1 hour. The positive

electrode was then washed in the same buffer and the power switched off. It was found that nucleic acids from the blood had been separated from the liquid and bound to the positively charged electrode and the DNA was then released into a working buffer ready for PCR.

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Claims

1. A method for separating nucleic acids from liquid mixtures containing them which method comprises forming a positively charged surface in the liquid and forming an electric field through the liquid to cause nucleic acids to migrate towards the positive charged surface and binding the nucleic acids to the positively charged surface.

2. A method as claimed in claim 1 in which an electric field is generated through the liquid containing the nucleic acids from outside the vessel containing the liquid.

3. A method as claimed in claim 1 which method comprises passing an electric current through the liquid to form a positively charged surface in contact with the liquid and to form an electric field through the liquid.

4. A method as claimed in claim 3 in which the electric current is generated between electrodes in contact with the liquid containing the nucleic acids.

5. A method as claimed in claim 4 in which the positive electrode is made from a conductive plastics material

6. A method as claimed in claim 5 in which the conductive plastics materials comprises an inert plastics material containing conductive particles.

7. A method as claimed in any one of the preceding claims in which a conductive material is incorporated in a component through or across which the liquid containing the nucleic acids is passed and the conductive material forms the positive surface.

8. A method as claimed in any one of the preceding claims in which a conductive material is incorporated in a pipette tip which conductive material comprises the electrode.

9. A method as claimed in claim 8 in which two pipettes are placed in a liquid containing nucleic acids in a vessel or tube and an electric current is passed between the pipettes so the nucleic acids become bound to the positively charged pipette tip.

10. A method as claimed in claim 8 in which the pipette is placed in a liquid containing nucleic acids in a vessel or tube and an electric current is passed between the pipette as the cathode and there is an anode spaced apart from the pipette tip so the nucleic acids become bound to the positively charged pipette tip.

11. A method as claimed in any one of claims 1 to 6 in which a conducting material is included in a PCR tube or plate which is made positive relative to a negative electrode or tip dipping in the solution containing the nucleic acids and the nucleic acids bind to the tube walls or plate.

12. A method as claimed in any one of the preceding claims in which the nucleic acid is separated from cells and in which a sample containing the cells is diluted in a lysing reagent that releases the nucleic acids and the current or electric field is then applied through the liquid so formed.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 41219 A (SCIENT GENERICS LTD ;WATSON SUSAN (GB); MARTIN SOPHIE ELIZABETH VI) 6 November 1997 (1997-11-06) page 1, line 15 -page 2, line 34 claims 1-6; examples 1,2 ---	1-12
X	WO 97 08293 A (SCIENT GENERICS LTD ;BERGMANN KARIN (GB); MARTIN SOPHIE ELIZABETH) 6 March 1997 (1997-03-06) claims 1-21; figures 1,5 ---	1-12
X	US 5 733 442 A (SHUKLA ASHOK K) 31 March 1998 (1998-03-31) column 1, line 10 - line 15 column 2, line 50 -column 4, line 26; claims 1-13; figures 1,8 --- -/--	1-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 34908 A (LANGE HANS ;INNOVA GES ZUR ENTWICKLUNG UND (DE)) 25 September 1997 (1997-09-25) claims 1-41; figure 1; examples 5,6	1-12
A	US 5 422 272 A (PAPP ANDREW A ET AL) 6 June 1995 (1995-06-06) column 2, line 30 - line 53; figures 1,2,7	8-10

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte onal Application No
PCT/GB 00/02842

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9741219 A	06-11-1997	EP 0904364 A	31-03-1999
WO 9708293 A	06-03-1997	CA 2230375 A	06-03-1997
		EP 0846161 A	10-06-1998
		JP 11511322 T	05-10-1999
US 5733442 A	31-03-1998	US 5340449 A	23-08-1994
WO 9734908 A	25-09-1997	DE 19610354 C	20-11-1997
		EP 0891369 A	20-01-1999
		US 6071395 A	06-06-2000
US 5422272 A	06-06-1995	NONE	